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Color Discrimination after Optic Nerve Regeneration in the Fish *Astronotus ocellatus*[1]

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INTRODUCTION

It has been shown that regeneration of the completely divided optic nerve in fishes and amphibians leads to an orderly recovery of the directional aspects of visual perception and that this occurs under experimental conditions which rule out learning and other functional types of readjustment (Sperry, 1942, 1943, 1944, 1948, 1955, 1958). It has been inferred from these findings that the fibers of the optic nerve must differ among themselves in quality, the individual axons being chemically tagged, so to speak, according to the exact location of their ganglion cell bodies in the retinal field. A similar specification of the tectal neurons is also suggested, such that each point in the retina has a matching point in the optic tectum.

Presumably, these refined specificities enable the scrambled optic fibers to untangle themselves and to achieve an orderly regrowth to their respective end stations in the tectum, thus restoring the original topographic projection of the retina on the optic lobe. This interpretation has received some support from postregenerative mapping studies involving localized tectal lesions (Sperry, 1944) and electrical recording of the tectal potentials evoked by retinal stimulation (Gaze, 1959; Maturana *et al.*, 1959). Direct microscopic confirmation of the selective reconnection has come recently from histological studies of optic nerve regeneration in goldfish and cichlids (Attardi and Sperry, 1960; Arora and Sperry, 1962).

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Additional problems in optic nerve regeneration arise in animals that have color vision, as do many of the fishes. The retina in these animals may contain specific photoreceptors for red and green and for blue and yellow, and others for luminosity (Svaetichin and MacNichol, 1958). Optic fibers differentially related to these various types of receptors would presumably each have to regain their respective kinds of central associations in the course of regeneration in order to effect recovery of normal color perception. An orderly recovery of color vision, such as is demonstrated in the present experiment, would thus seem to require the presence of additional dimensions of specificity among the optic fibers over and above those associated with directionality.

MATERIALS AND METHODS

Animals. The freshwater cichlid *Astronotus ocellatus,* known commonly as the "Oscar" or "velvet fish," was used throughout this investigation. Although native to the Amazon basin, the species is widely available from tropical fish dealers in the United States and elsewhere. The fish is predaceous and depends largely on sight for the capture of food. It also is an inhabitant of shallow water, another factor favoring the evolution of good color vision. The adult attains a length of approximately 25 cm. Only young specimens ranging in total length from 4 to 9 cm at the start were used in the present study.

Surgical procedure. The surgical preparation, anesthetization, and postoperative treatment were the same as described in earlier studies (Arora and Sperry, 1957) with the exception that a stronger concentration of anesthetic (i.e., 0.15 gm of Tricaine methanesulfonate per liter of water) was used for fish 6 cm or longer, requiring immersion for 5–8 minutes. The optic nerve was sectioned in the orbit through a dorsal incision in the conjunctiva. A transverse slit was made in the transparent sheath covering the optic nerve, after which the nerve was cut with iridectomy scissors. The two cut ends were mashed and teased apart with forceps until complete division and disarrangement of the nerve fibers could be seen. A small strand of the outer dural sheath was left intact to prevent excessive separation of the two stumps.

Behavioral training and testing. During training the fish were kept individually in 3-gallon tanks in conditioned tap water which was continually aerated and maintained at approximately 78°F by means of

thermostatically controlled heating elements. For the first week or so the fish were fed with forceps, first with the food held under water, then at the surface, and then at gradually increased elevations above the surface until the fish learned to jump out of water to a height of 1–3 cm in order to secure the food. The forceps were then replaced by the training apparatus described below, using first a single tube with either a neutral color or, in a few instances, with the positive color later to be rewarded. For 2 or 3 days the fish refused to approach the new feeder, but following this period of deprivation they began to snap at it and then jump out of water to obtain the attached food. Once the fish had overcome the fear of the new feeder, the second tube was introduced, thus presenting a choice between positive (rewarded) and negative (unrewarded) colors.

The training apparatus (diagrammed in Fig. 1) consisted essentially of two similar tube-shaped feeders set vertically in a connecting plate about 4 cm apart. The two feeders were distinguishable from below by different-colored lights, transmitted through monochromatic gelatin filters. The light source was a miniature 18-volt bulb fitted into the upper end of each tube with the brightness of each separately and continuously adjustable through two variable rheostats. Each tube was of copper 1 inch in diameter and 4 inches in length. The color filter,

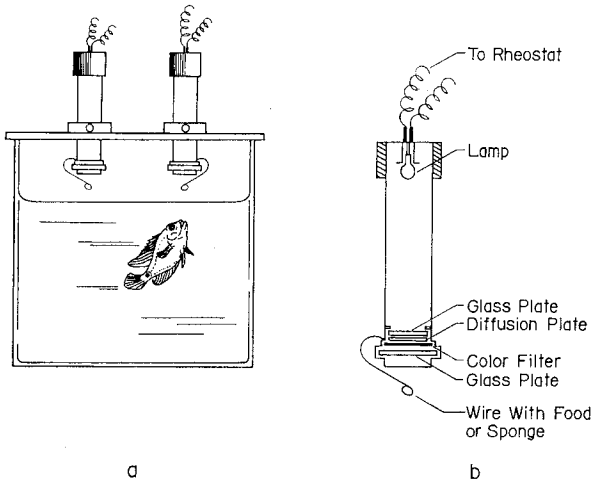

FIG. 1. Diagram of training apparatus. (a) Position of feeders during training. (b) Detailed construction of single feeder.

set near the lower end of the tube, was held by an adapter ring and protected by a thin glass plate from the heat of the lamp. Kodak diffusion film plate was placed just behind the glass plate to ensure more even distribution of the light. The lower end of the tube was closed with a protecting glass disk.

The setting of the tubes in the connecting aluminum plate could be adjusted both horizontally and vertically. All portions of the apparatus below the aluminum plate were painted black, including the lower surface of the plate itself. The filters used were Kodak wratten gelatin of the tabulated specifications.

Catalog no.	Visual color	Spectral transmission
47	Blue	370–510 mμ
58	Green	480–620 mμ
90	Yellow	560–630 mμ and infrared
92	Red	630 mμ into infrared
96	Neutral	Densities varied from 0.10 to 2.00

The two tubes, positive and negative, were placed parallel to the end of the aquarium tank and 1–2 cm above the surface of the water. A morsel of food was impaled on the tip of a stainless steel wire directly under the colored end of the positive tube; a bit of sponge similar in appearance was placed on the negative tube. During training the sides of each tank were wrapped with opaque paper to lessen distractions and also to keep the observer unaware of the starting posture of the fish as the feeders were set into position. Right-left positioning of the feeders was varied on a prearranged pseudorandom schedule. The colored filters and also the adapter rings inside the feeder tubes were interchanged at intervals in order to rule out extraneous cues in the training apparatus. Each fish was given 10 trials twice a day, the room being kept dimly lit during the training.

OBSERVATIONS

Color Discrimination before Operation

The ability to perceive color was first verified by training 12 normal fish to select a correct (rewarded) color when it was paired with other colors and shades of gray. Red, blue, green, and yellow were each used as the rewarded color in different cases. In 40–60 trials

after introduction of the double feeder the fish had begun to choose the positive color, and by 120–140 trials they were making 9 correct out of 10 or better. The sample learning curves shown in Fig. 2 are typical. The original negative color was then replaced in turn by grays of various shades and by other colors, until after roughly 200 trials the fish were consistently selecting the positive color when it was paired with any of the other colors mentioned above or with any shade of gray. A series of from 10 to 20 critical trials were then run in which meat was impaled on both the positive and negative feeders. All the fish continued under these critical conditions to select the correct color.

In further exploratory tests some extra trials were run with the various colors presented singly. In this case the fish, which by now had been thoroughly trained, continued to jump at the single feeder when it had the correct color, but they refused to jump at any of the negative colors. Further, the fish refused to jump at either of two different shades of gray when these were presented simultaneously on the double feeder. Exceptions to the foregoing were observed when the fish were abnormally excited by hunger or other factors, but the responses for the most part were convincingly consistent.

Color Vision after Optic Nerve Regeneration

In four untrained specimens, both optic nerves were sectioned, leaving the fish totally blind. By about the 26th day following the operation the fish showed signs of returning vision, in the form of alerting reactions made to objects moving near the tanks. By the 36th to 40th day recovery appeared to be complete. The fish would follow and seize small bits of food sinking slowly in the water, responding with apparently normal accuracy. The fish would also approach correctly and follow bits of food impaled on a fine wire when these were presented and moved about outside the glass wall of the tank. These four fish with recovered vision were then trained to discriminate colors in the same manner as described above, including the controls for shades of gray. Their learning curves did not differ significantly from those of the normal, unoperated fish.

Restoration of Preoperatively Trained Color Discriminations

Finally, there remained the question whether colors are perceived in their original form following optic nerve regeneration. To check this

point, color discriminations that had been trained before sectioning of the optic nerve, were tested again following regeneration, under conditions that excluded relearning. This procedure was carried out with a group of twenty fish. After training and overtraining to a total of 200–300 trials, the optic nerve on one side was cut. The trained performance was further reinforced through the normal eye for another 40–100 trials during the next 14–18 days. The second optic nerve was then cut, resulting in total blindness for a period of 7 to 10 days. Visual recovery on the side of the first operation appeared to have been fully established by 35 to 40 days after the first nerve had been sectioned.

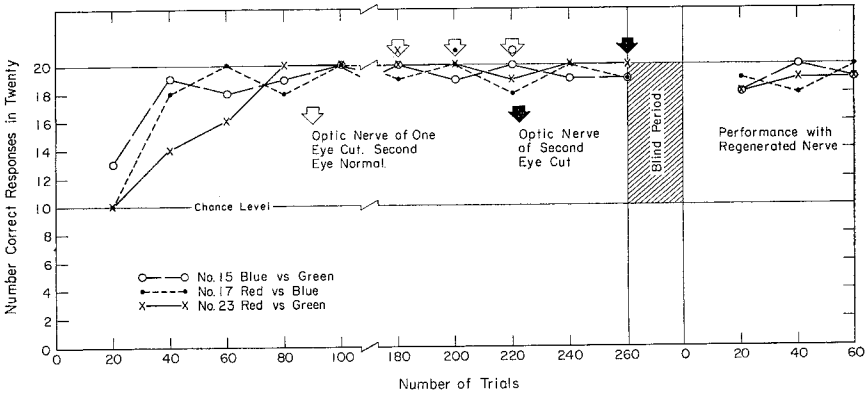

FIG. 2. Sample learning curves for three fish including performance following section and regeneration of optic nerve. Trials between 100 and 180 (overtraining and testing for extraneous cues) have been omitted.

Critical testing for retention of the pretrained color preferences was started on or after the 40th day. In the first 10 of these critical trials both feeders were baited similarly, that is, both with food or both with sponge on a prearranged erratic schedule, and the color filters were interchanged on the sixth trial. The brightness of each color, positive and negative, in these first 10 trials was kept constant at a moderate level and closely equated in the experimenter's judgment. In the next 10 trials both the positive and the negative feeders were similarly baited with food, but the brightness of the negative color was erratically shifted from trial to trial, varying from markedly above to well below that of the positive stimulus.

Nineteen of the 20 cases obtained 18 or more correct responses in these first 20 trials following return of vision. The remaining case made 15 out of 20 correct responses. In further control testing the performance of this group was similar to that of the normal, unoperated group described earlier, i.e., the fish with regenerated optic nerve refused to jump to negative colors presented singly or to either of two shades of gray. The sample learning and test curves of the 3 cases shown in Fig. 2 are representative of the group.

DISCUSSION

It seems evident from the foregoing that color vision is subject to orderly recovery in optic nerve regeneration. Gross color differences as between red, yellow, green, blue, and gray were distinguished after regeneration, and responses to each color were the same as before nerve section, indicating that each of the colors regained its own identity. To what degree the discrimination threshold for fine shades of color difference may be restored remains to be tested.

In any case it would appear that the optic axons that pick up impulses from the different types of photoreceptors must establish synaptic relations in the tectum suited in each case to the particular receptor type or combination involved. If fibers activated by red light were to form the same kind of functional relations centrally as those for blue, yellow, or green light, the various colors would not be distinguishable except in terms of discharge frequency or other differential effects carried by the same axon. The physiologic evidence in general (Wagner *et al.*, 1960; De Valois, 1960) seems to allow some coding of the latter kind, but it also favors the existence of a basic set of specific fiber types differentially reactive to the primary colors.

This means that a given optic axon must be qualitatively tagged not only for the latitude and longitude of its destined synaptic zone in the tectum, but also for the kind of color information it will carry. Different fibers arising from the same retinal locus and terminating in the same tectal zone must synapse selectively and specifically according to the individual pattern of bipolar-photoreceptor associations in the retina. This reasoning applies as well to the "on," "on-off," and "off" fiber types in the optic nerve (Hartline, 1938) and to the "convexity," "edge," and other detector types as listed by Lettvin *et al.* (1959) and Maturana *et al.* (1959, 1960). The optokinetic, pursuit,

and object-discrimination responses that have been observed to undergo an orderly restoration in optic nerve regeneration could hardly have done so if the various types of optic fibers had become mixed and failed to form each its own appropriate central associations. Electrical evidence supporting selective reconnection of this kind has been reported for the frog (Maturana *et al.*, 1960).

In the same way that the cones become differentiated from the rods in embryonic development and also differentiate among themselves into several types selectively sensitive to different colors, so we may infer also that the bipolar and ganglion cells become differentiated into a number of specific types differing in physiological, biochemical, and in some cases morphological properties. Also we must conclude that a similar central differentiation takes place among the receiving neurons of the optic tectum and on a corresponding order of complexity.

Given such a qualitative specification among the optic fibers on the one hand and among the tectal neurons on the other, it is easy to understand how the appropriate synaptic connections between the two could then be restored in accordance with the author's (Sperry, 1943, 1958) chemoaffinity theory of synaptic formation. Not all the optic axons connect in the tectum; some go to the lateral geniculate nucleus and others to less defined centers of thalamus and midbrain. Similar selective affinities may be presumed to operate in regulating synapsis among these extratectal components as well.

Application of the foregoing interpretation to the mammalian visual system meets certain complications relating to the evolution of the geniculostriate structure and the double projection of visual half-fields to each midbrain colliculus. These mammalian developments, however, do not contradict the theory but demand additional refinements. For example, the evolution of some factor or factors must be postulated that would cause fibers from the temporal retinas to turn ipsilaterally instead of crossing. The radial or foveociliary gradient of the mammalian retina must feature strongly in getting the nasal hemiretina of one eye to project together and in register with the temporal hemiretina of the other eye. Further details must be added to account for the layering of the lateral geniculate bodies and the ordering of their cortical projections. All these fall within the scope of the general interpretation, however, and indeed it would be difficult to account

for the known anatomical organization of the visual system in the absence of regulative phenomena of the sort proposed.

SUMMARY

Fish were trained to jump at the correct (rewarded) one of two differently colored feeders and to avoid feeders of other colors and different shades of gray. It was found that the fish could discriminate the colors red, blue, yellow, and green both in the normal state and after complete section and regeneration of the optic nerve.

When the color preferences were trained prior to optic nerve section, regeneration of the nerve resulted in reinstatement of the same color preferences, no relearning being necessary. Some of the implications regarding specificity of the optic fibers and factors governing their central synapsis in the optic tectum are discussed.

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